Journal of the American Mosquito Control Association, 14(2):183–185, 1998

LABORATORY AND FIELD EVALUATION OF EFFICACY OF VECTOBAC®12AS AGAINST *CULEX SITIENS* (DIPTERA: CULICIDAE) LARVAE

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ABSTRACT. Laboratory bioassay studies of the efficacy of VectoBac®12AS (active ingredient: 1,200 International Toxic Units [ITU]/mg *Bacillus thuringiensis* var. *israelensis*) against field-collected late 3rd/early 4th-instar larvae of *Culex sitiens* indicated excellent control potential. A 95% lethal concentration (LC_{95}) value of 1.381×10^7 ITU was calculated, which equated to a dosage of 0.011 liters/ha. This dosage represented 1.8% of the recommended lowest dosage rate for the product. A field trial of VectoBac 12AS against late 3rd/early 4th-instar field specimens of *Cx. sitiens* in floating mesh cylinders was then conducted in salt-marsh pools near Coomera Marina, southeast Queensland, Australia. At a rate of 0.5 liters/ha, 100% mortality of *Cx. sitiens* larvae was recorded at 24 h posttreatment.

KEY WORDS *Culex sitiens, B.t.i.,* pesticide, larvicide

INTRODUCTION

In Queensland, Australia, *Culex sitiens* Weidemann is an important pest species that utilizes intertidal pools as a larval habitat (Lee et al. 1989). As a consequence of environmental concerns over temephos usage (Mortimer and Chapman 1995, Brown et al. 1996), control personnel have been evaluating alternate larvicides for the elimination of this mosquito.

Foreign evaluations of various formulations of *s*-methoprene (Sawby et al. 1992; Ross et al. 1994a, 1994b) and *Bacillus thuringiensis* var. *israelensis* (*B.t.i*) (Margalit and Dean 1985, Gharib and Hilsenhoff 1988, Lee et al. 1996) have shown that these products can control mosquitoes with minimal environmental impact. Unfortunately, recent evaluations of *s*-methoprene against Australian mosquitoes (Ritchie et al. 1997), have shown that *Cx. sitiens* tolerates extremely high levels of this product. A 99% lethal concentration (LC_{99}) value of 18 ppb *s*-methoprene was calculated for *Cx. sitiens*. This suggests that at the label rate, *s*-methoprene treatments will fail from March through May when *Cx. sitiens* is most abundant in salt-marsh habitats in southeastern Queensland. Accordingly, we conducted laboratory and field investigations into the efficacy of a liquid formulation of *B.t.i.* for use against *Cx. sitiens* larvae.

MATERIALS AND METHODS

Laboratory bioassays: Laboratory bioassays, based on standard methods for testing of larval susceptibility (World Health Organization 1981), were used to determine the effectiveness of VectoBac®12AS (active ingredient: 1,200 International Toxic Units [ITU]/mg *B.t.i.* [1.279×10^9 ITU/liter], with a label rate of 0.6–1.2 liters/ha, Hoechst Schering AgrEvo Pty. Ltd., Pennant Hills, New South Wales, Australia) against field-collected *Cx. sitiens* larvae. The larvae were collected from salt-marsh pools near Coomera Marina (27°54′S, 153°17′E). Habitat water was also collected for experimental and maintenance purposes. In the assays, larvae were exposed to serial dilutions of VectoBac 12AS in filtered (100-μm-mesh net) habitat water. Five replicates each of 20 late 3rd/early 4th-instar larvae were introduced into 250-ml glass beakers containing 200 ml of test concentration. Five control beakers holding 20 test larvae each in habitat water without pesticide were used for each bioassay. To minimize variability due to nutritional and metabolic condition, larvae were not fed for 24 h prior to or during testing. Initially, a number of range-finding bioassays with widely spread exposure concentrations were conducted. Based on these tests, a narrow range of concentrations that straddled the effective range were conducted. The numbers surviving were counted at 24 h. Death or the lack of reaction to gentle prodding with a glass pipette was the measured mortality. The assays were conducted at 25°C under a light : dark cycle of 12:12 hours.

Field bioassays: The laboratory bioassay data indicated that effective *Cx. sitiens* control could be achieved at an application rate of 0.5 liters/ha. Accordingly, the bioefficacy of this dosage was evaluated in the field in conjunction with the currently utilized dosage rate of 1.0 liter/ha. The field trial was conducted by exposing field-collected late 3rd/early 4th-instar *Cx. sitiens* larvae to 0.5 liter/ha concentrations of VectoBac 12AS in small (ca. 5-m^2, 5–10-cm-deep) salt-marsh pools near Coomera Marina. The formulation was applied using a pressurized knapsack sprayer (Stainless Steel Tanks and Pressure Vessels Pty. Ltd., Melbourne, Victoria, Australia). Assessment of efficacy was made by the use of floating 20-cm-deep × 100-cm-diam, open-

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Table 1. Twenty-four-hour probit analyses of VectoBac® 12AS toxicity to 3rd-instar *Culex sitiens.*[1]

LC_{50}[2] (liters/ha)	LC_{95}[2] (liters/ha)	Intercept (SE)	Slope (SE)	P[3]	Abiotic characteristics (mean ± SD) pH	Turbidity (NTUs)	Dissolved oxygen (mg/liter)	Temperature (°C)	Salinity (ppk)
0.0077 (0.0074, 0.0081)	0.011 (0.010, 0.0120)	42.485 (4.759)	20.125 (2.251)	0.983	7.38 (±0.13)	0	3.86 (±0.3)	25	25

[1] LC_{50}, median lethal concentration; LC_{95}, 95% lethal concentration; NTUs, nepholometric turbidity units.
[2] Values in parentheses are the 95% lower and upper confidence limits.
[3] P refers to the probability corresponding to maximum likelihood chi-square statistic for goodness of fit of the model.

topped, 280-μm mesh cylinders. Six intertidal salt-marsh pools were selected for study, and one cylinder containing 100 *Cx. sitiens* was placed in each pool 2 h prior to treatment. Of these, 3 pools were selected as untreated controls and 3 pools received a 0.5 liter/ha treatment. Control pools were located upwind of those treated with *B.t.i.* The numbers surviving were counted at 24 h, and percentage mortality was calculated. Because abiotic parameters of water can affect the toxicity of a substance (Cooney 1995), temperature, pH, dissolved oxygen (DO), salinity, and turbidity were recorded prior to treatment and at 24 h using a portable field laboratory (Horiba Ltd., Kyoto, Japan).

Analysis of data—laboratory bioassay: A probit model was used to analyze *Cx. sitiens* mortality as a function of *B.t.i.* concentration. The SPSS for Windows version 7.0 PROBIT procedure (Norusis 1990) was used for this analysis. The median lethal concentration (LC_{50}) and 95% lethal concentration (LC_{95}) and associated 95% confidence intervals were calculated.

Analysis of data—field bioassay: Repeated measures of analysis of variance models were used to test the significance of pre- to postapplication changes in each of the abiotic water variables. Poisson regression models were then used to consider whether the mean postapplication larval count differed by treatment group for any of the abiotic water variables. Repeated measures analysis of variance models were used to model postapplication larval counts by 0.5 liter/ha dose of VectoBac®12AS, allowing estimates of effect to be adjusted for any changes in the abiotic water variables.

RESULTS AND DISCUSSION

From this study it is evident that *C. sitiens* is extremely sensitive to *B.t.i.* In the laboratory bioassay study, an LC_{95} value of 0.011 liters/ha was calculated, which represented 1.8% of the low label rate for VectoBac 12AS in Australia (Table 1). This sensitivity may have been augmented in the laboratory bioassays by a number of factors. The absence of food in the test containers, the 24-h starvation period prior to testing, and the absence of particulate matter (turbidity of 0 nepholometric turbidity units [NTUs]) other than VectoBac 12AS, which was the only available material during testing, may all have contributed to this result.

Consequently, in the field *Cx. sitiens* suffered 100% mortality at both 0.5- and 1.0-liters/ha concentrations of VectoBac 12AS. No statistical evidence was found to suggest that posttreatment larval counts were associated with any of the environmental variables ($P > 0.115$). These results are encouraging, as they mean an effective alternative to temephos and *s*-methoprene is available for treatment of *Cx. sitiens* larvae, given appropriate formulation.

During the field study, significant posttreatment changes in turbidity ($P < 0.013$), temperature ($P < 0.001$), salinity ($P < 0.001$), and depth ($P < 0.001$) were recorded. Changes in DO ($P < 0.055$) and pH ($P < 0.091$) were not statistically significant. No statistical evidence was found to suggest that these changes were associated with the VectoBac 12AS treatments ($P > 0.190$) (Table 2).

From October through February in southeastern Queensland, *Aedes vigilax* (Skuse) is the predomi-

Table 2. Abiotic water characteristics of salt-marsh pools 24 h after treatment with VectoBac® 12AS.[1]

Application rate	EFC (ITU/ha)	Mean (±SD) abiotic water characteristics in control and treatment pools pH	Turbidity (NTUs)	Dissolved oxygen (mg/liter)	Temperature (°C)	Salinity (ppk)
Control	0	9.3 (±0.5)	35 (±6)	3.77 (±0.88)	32.9 (±0.2)	27.1 (±4.5)
0.5 liters/ha	0.64×10^9	9.2 (±0.3)	57 (±18)	3.77 (±1.12)	33.7 (±0.8)	25.0 (±3.9)
1.0 liters/ha	1.28×10^9	9.4 (±0.2)	51 (±11)	4.25 (±0.96)	34.7 (±0.4)	27.2 (±1.8)
P		0.355	0.285	0.849	0.0631	0.697

[1] EFC = estimated field concentration; ITU, international toxic units; NTUs, nepholometric turbidity units.

nant salt-marsh mosquito requiring control. *Culex sitiens* then becomes the major problem from March through May. Accordingly, given suitable formulations of *B.t.i.*, a program in which rotation of *s*-methoprene followed by *B.t.i.* from March onwards when *Cx. sitiens* are most plentiful would seem a sensible option. Also, in terms of acute toxicity, Vectobac 12AS has been proven safe for use with cohabiting shrimps (*Leander tenuicornis* Say) and Pacific blue-eye (*Pseudomugil signifer* Kner) fish (Brown et al. 1996, Brown et al. submitted). Accordingly, we believe the calibration of land-based and air-borne ultra-low volume equipment at 0.5 liters/ha is sufficient for applications against *Cx. sitiens* larvae in salt-marsh pools in southeastern Australia. This still leaves a safety margin of 45 times the LC_{95} to guard against uneven applications.

ACKNOWLEDGMENTS

We thank the technical officers representing the Local Authorities Research Committee for their support and encouragement. Harry Standfast (International Vector Consultants) and Peter Ryan (Queensland Institute of Medical Research [QIMR]) provided useful discussion and guidance. Diana Battistutta (Medical Biostatistics Pty. Ltd.) was responsible for statistical analysis. Paul Mason (Gold Coast City Council) and Kay Marshall (QIMR) provided technical assistance.

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